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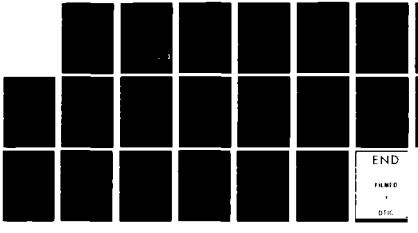

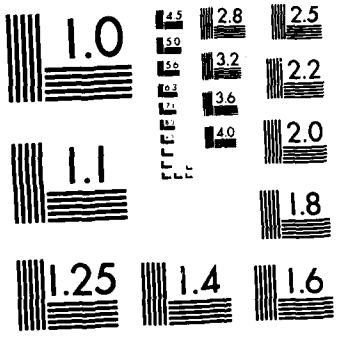

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OVIPOSITION-MODIFYING SUBSTANCES FOR MOSQUITOES

Annual Summary Report

YIH-SHEN HWANG

September 1, 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Fort Detrick, Frederick, Maryland 21701

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Department of Entomology, University of California

Riverside, CA 92521

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MOSQUITOES/OVIPOSITION-ATTRACTANTS/OVIPOSITION-REPELLENTS/OVIPOSITION-MODIFYING-SUBSTANCES/CARBOXYLIC-ACIDS/OCTANOIC-ACID/NONANOIC-ACID/ORGANIC-INFUSIONS/Culex-quinquefasciatus/Culex-tarsalis/Culex-peus

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

The objectives of this research project are to study the chemistry and the biology of oviposition-modifying substances for mosquitoes, to investigate the possibility of applying them in the management of mosquito populations, and to evaluate the role of oviposition attractants in sampling populations of female mosquitoes and ovipopulations.

The study on the structure-activity relationship of homologous, straight-chain

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aliphatic carboxylic acids as oviposition repellents revealed that octanoic, nonanoic, and decanoic acids were the most active against gravid females of Culex quinquefasciatus, Cx. tarsalis, and Aedes aegypti. Other types of compounds, such as skatole, 1-hexadecanol, and 2-methylnonanoic acid, were also repellent against Cx. quinquefasciatus.

The semi-field evaluation of oviposition repellents in experimental field ponds with the use of metal-sheet cylinders demonstrated that octanoic acid, at the 15 and 30 ppm concentrations, suppressed gravid female mosquitoes from ovipositing. At a lower concentration, this acid did not show any repellency.

The full-scale field evaluation of oviposition repellents in experiment field ponds showed that octanoic acid, at 10, 20, 25, and 50 ppm, failed to prevent gravid female mosquitoes from ovipositing in the ponds. However, nonanoic acid was able to repel ovipositing females for about two weeks at the 150 ppm concentration.

Chicken manure infusions, which had been shown to possess ovipositional attractancy in laboratory olfactometers, attracted Cx. tarsalis and Culiseta inornata for oviposition under field conditions.

In the isolation and identification of oviposition attractants from the chicken manure infusions, the infusions were steam-distilled to give an attractive distillate which, upon extraction with ether, yielded an active ether extract. Fractionation of the extract produced a repellent basic fraction, an inactive acidic fraction, and an attractive neutral fraction. Purification of the neutral fraction with column chromatography on silica gel gave a major component which showed ovipositional attractancy. Tlc and glc methods were used to determine the purity and the composition of these fractions. Spectrometric techniques, such as ir, nmr, and ms, were employed to analyze this major component of the neutral fraction.

SUMMARY

The objectives of this research project are to investigate the chemistry and the biology of oviposition-modifying substances for various species of mosquitoes, to study the possibility of applying these substances in the management of mosquito populations, and to evaluate the role of oviposition attractants in sampling female adults and in determining their ovipositional activity. Previously, we conducted the isolation and identification of mosquito oviposition repellents, biologically characterized them, studied their concentration-activity relationship and species specificity, and investigated their toxicity against immature mosquitoes. The location of chemoreceptors that mediate oviposition repellents was also identified.

During the past funding year, we placed our emphasis on (1) the structure-activity relationship and the species specificity of a series of homologous, aliphatic carboxylic acids which showed oviposition repellency, (2) the semi-field evaluation of oviposition repellents, (3) the field evaluation of oviposition repellents, (4) the field evaluation of attractive organic infusions, and (5) the isolation and identification of oviposition attractants.

The study on the structure-activity relationship of homologous, straight-chain, aliphatic carboxylic acids from C_5 to C_{13} as oviposition repellents revealed that octanoic, nonanoic, and decanoic acids were, in general, the most active against gravid females of Culex quinquefasciatus, Cx. tarsalis, and Aedes aegypti. The level of repellency was about the same among these three species of mosquitoes. The active ranges of these compounds were about 10^{-2} to 10^{-4} M. Other types of compounds, such as skatole, 1-hexadecanol, and 2-methylnonanoic acid, were also repellent against Cx. quinquefasciatus.

The semi-field evaluation of oviposition repellents was conducted by using metal-sheet cylinders in experimental field ponds. Water confined in the cylinders was first spiked with attractive chicken manure to increase mosquito oviposition and then treated with repellent octanoic acid. We found that those cylinders containing 15- or 30-ppp octanoic acid received less mosquito oviposition than the control cylinders for a period over three weeks. At a lower concentration, this carboxylic acid did not show measurable repellency under semi-field conditions.

The full-scale field evaluation of oviposition repellents was carried out in experimental field ponds. Octanoic acid, dissolved in the 1:1 (w:v) ratio with either 1-propanol or 2-propanol, was used as an oviposition repellent. In the first two experiments, we found that the ponds containing octanoic acid at 10, 20, 25, and 50 ppm received as many ovipositions as the control ponds. This failure of repelling mosquitoes from oviposition might have been due to the uneven distribution of the repellent and its quick dissipation through evaporation and absorption by the soil. However, nonanoic acid, formulated with 1-propanol and Triton X-100, was able to repel ovipositing female mosquitoes for about two weeks at the 150 ppm concentration. More experiments for the field evaluation of the repellents are being conducted to determine the lowest effective concentration for repelling mosquitoes from oviposition.

Chicken manure infusions, which had been shown to possess ovipositional attractancy in laboratory olfactometers, were applied in the experimental field ponds to evaluate their efficacy in attracting ovipositing female mosquitoes under field conditions. Over the three-week period, the ponds treated with 1% chicken manure collected more eggs than the control ponds. Mosquitoes oviposited in the ponds during the period of this experiment were mainly Cx. tarsalis with a small number of Culiseta inornata.

In the isolation and identification of oviposition attractants from the chicken manure infusions, the infusions were steam-distilled under atmospheric pressure to give an attractive distillate which, upon extraction with ether, yielded an active ether extract. Fractionation of the ether extract produced a repellent basic fraction, an inactive acidic fraction, and an attractive neutral fraction. Purification of the neutral fraction with column chromatography on silica gel gave a major component which showed ovipositional attractancy in laboratory olfactometers. Thin-layer and gas chromatographies were used to study the compositions of these fractions. Infrared, nuclear magnetic resonance, and mass spectrometric techniques were employed to determine the chemical structures of the oviposition attractants.

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ANNUAL REPORT

A. INTRODUCTION

Mosquitoes breed in a variety of water accumulations; however, each species is adapted to a characteristic type of habitat. Intensive field studies have shown that the distribution of mosquito larvae is controlled not only by their survival potential in suitable habitats but also by the selective discrimination of the ovipositing females in locating oviposition sites. It is, therefore, most likely that gravid female mosquitoes of different species use different physical and/or chemical factors as cues in selecting oviposition sites. The influence of various physical factors on the selection of oviposition sites was reviewed and discussed by Clements (1963). The chemical factors may consist of oviposition pheromones and oviposition attractants and repellents. Oviposition pheromones may occur in nature as intraspecific messengers to inform conspecifics of suitable oviposition sites. Oviposition attractants and repellents are generally believed to be produced in nature by microbial fermentation and breakdown of organic matter, and these transspecific messengers serve as kairomones or allomones in providing cues for gravid mosquitoes to detect suitable or unsuitable oviposition sites. If these oviposition-modifying substances become known and available to us, they can be employed in measuring mosquito populations and used in manipulating mosquitoes through the regulation of mosquito oviposition. Thus, these substances offer a potential as supplementary measures for mosquito control. Additionally, a knowledge of these behavior-modifying substances could provide a basis for understanding behavioral responses of gravid mosquitoes which constitute an important portion of the total populations.

B. BACKGROUND

The presence of chemical attractants and repellents in natural habitats for mosquito oviposition has been demonstrated by many researchers. Gerhardt (1959) reported that ovipositing female mosquitoes were attracted by stimuli, acting on their olfactory receptors and directing them to oviposition sites containing excess organic matter. In qualitative studies, Gubler (1971) showed that ovipositing females of Aedes (Stegomyia) albopictus Marks and Ae. (S.) polynesiensis Marks were repelled by high concentrations of ammonia and protein solutions, but attracted by high concentrations of organic infusions such as leaf, grass, and guinea pig chow. Gjullin et al. (1965) reported that grass infusions and log pond water increased oviposition of Ae. aegypti L. and Culex quinquefasciatus Say. Ikeshoji (1966a,b, 1968) showed the existence of oviposition attractants for Cx. fatigans Wiedemann in natural breeding water and attempted to isolate and identify them. Crude mixtures of oviposition attractants for Cx. quinquefasciatus, Cx. tarsalis Coquillett, Ae. nigromaculis (Ludlow), and Ae. taeniorhynchus (Wiedemann) were isolated from natural breeding water by Ikeshoji and Mulla (1970). Several workers produced evidence showing that bacteria produced certain oviposition attractants and stimulants as degradation products of organic matter (Hazard et al. 1967, Maw 1970, Ikeshoji et al. 1975).

From the evidence in the literature and from our own preliminary investigations, it is evident that, in natural breeding sites, microbial decomposition of certain organic matter produces volatile substances which act as oviposition-modifying factors for various mosquitoes. These substances may be species-specific or non-specific. They can be attractants, repellents, stimulants, or deterrents.

In view of these successful approaches to produce mosquito oviposition-modifying substances in infusions of organic matter, we initiated exploratory studies on the biology and chemistry of some fermentation products of organic matter showing oviposition-modifying activities. After investigating infusions of various organic substrates, we found that a 1% lab chow infusion was significantly repellent to several species of ovipositing female mosquitoes and that a 1% chicken manure infusion was significantly attractive to Cx. quinquefasciatus females but repellent to Cx. tarsalis females (Kramer and Mulla 1979). These infusions were prepared by adding 1 part of lab chow or chicken manure in 100 parts of tap water and allowing the resulting suspensions to ferment at room temperature. The lab chow infusion became repellent after five days and stayed active for two more weeks. The chicken manure infusion showed attractancy from 9 to 26 days after the start of fermentation.

For carrying out a number of different chemical and biological investigations, a large amount of stock infusions was needed. It thus became necessary for us to determine the storage stability of the infusions at various temperatures. Our studies showed that both infusions remained active for ten weeks or longer when stored in a freezer at -10°C. This storage technique has enabled us to store a large quantity of active infusions for considerable period of time. The storage stability was short-lived at 10° or 20° C.

For characterizing the response of mosquitoes to the oviposition repellents, the effects of several physiological parameters on the responses of mosquitoes were investigated. We found that the interval between blood feeding and oviposition, the parous condition, and the prior exposure to test infusions in bio-assays did not affect the response of gravid mosquitoes to the 1% lab chow infusion.

As previously described, the infusions were not active when freshly prepared, but they gradually became active as the aging process proceeded. It was suspected that bioactive compounds were produced as a result of microbial fermentation. The involvement of microorganisms in the production of attractants and repellents was determined by comparing the activity of the infusions prepared and brewed under septic and aseptic conditions. The aseptic infusions remained inactive whereas the septic infusions consistently showed repellency or attractancy (Kramer and Mulla 1979).

The production of active compounds by microorganisms in organic infusions was thus confirmed, and the biological activity of these compounds was characterized. Consequently, chemical investigations seemed to be in order for the determination and the structural elucidation of the active compounds which elicited positive or negative oviposition response in msoquitoes. In our studies on the isolation and chemical identification of the oviposition repellents, the active lab chow infusion was distilled to give an active distillate which, upon ether extraction, gave an active ether extract. Fractionation of the ether extract yielded an active acidic fraction and an inactive non-acidic fraction. Gas chromatographic analysis on Porapak R and AT-1200--H_3PO_4 columns of the acidic fraction showed the presence of acetic, propionic, isobutyric, butyric, isovaleric, and caproic acids; among these acids, butyric acid constituted 85% of the total amount of these acids. In bio-assay tests, these aliphatic carboxylic acids, individually and in combination, exhibited ovipositional repellency against the two species of Culex mosquitoes at the concentration of $6X10^{-2}$% (Hwang et al. 1978, 1980).

Further studies on the concentration-activity relationship of the oviposition repellents revealed that acetic and isobutyric acids were significantly repellent to Cx. quinquefasciatus at the $6X10^{-3}$% concentration and caproic acid was repellent to Cx. tarsalis at the same concentration (Kramer et al. 1980).

In our species specificity studies, butyric acid was repellent to Ae. aegypti and Culiseta incidens (Thomson) only at the very high concentration of $6X10^{-1}$% and to Anopheles quadrimaculatus Say at $6X10^{-2}$ and $6X10^{-3}$%. Butyric acid, however, at $6X10^{-2}$ and $6X10^{-3}$% significantly attracted Cs. incidens females for oviposition (Kramer et al. 1980). These results clearly demonstrated that butyric acid, as oviposition repellent, showed species specificity against various species of mosquitoes. It also acted as both repellent and attractant against Cs. incidens dependent upon its concentrations.

Butyric acid is, therefore, considered as a mosquito behavior modifier in our investigations. When this acid at $6X10^{-2}$% was exposed to gravid mosquitoes whose antennae and/or tarsi had been extirpated, only those mosquitoes with one or both antennae partially or totally extirpated ceased to respond to the acid. These findings have led to the conclusion that the chemoreceptors for the perception of butyric acid are located on the antennae.

C. OBJECTIVES

The objectives of this research project are:

1. To investigate the chemistry of oviposition-modifying substances for various species of mosquitoes.

The oviposition-modifying substances for mosquitoes will be isolated and purified from natural sources, and active compounds will be chemically identified or their structures will be elucidated. If the active compounds are novel and commercially unavailable, they will be synthesized. Analogues or homologues of the active compounds will also be synthesized for structure-activity studies.

2. To investigate the biology of oviposition-modifying substances.

Responses of various species of ovipositing female mosquitoes to the oviposition-modifying substances will be studied in the laboratory. The sensory physiology and the toxicity of these behavior-modifying substances will be investigated. The concentration-activity relationship and species specificity of these substances against various species of mosquitoes will be studied.

3. To study the possibility of applying oviposition-modifying substances in the management of mosquito populations.

To achieve this goal, these substances will be evaluated under semi-field and field conditions against various species of mosquitoes. This will allow us to assess the effectiveness of using the oviposition-modifying substances to manipulate ovipositional behavior of mosquitoes under field conditions.

4. To evaluate the role of oviposition attractants in sampling populations of female adult mosquitoes and ovipopulations.

The oviposition attractants will be evaluated in traps or light traps to assess their efficacy for collecting female adult mosquitoes and eggs for population and epidemiological studies. Mosquitoes that do not readily respond to conventional light traps may possibly be collected by this technique.

D. SPECIFIC AIMS

The specific aims of the present research program are:

1. To isolate and purify oviposition-modifying substances from infusions of various organic materials that possess oviposition-modifying activity by means of physical and chemical procedures in pure or semi-pure forms for chemical identification.

2. To chemically identify or to elucidate the structures of the oviposition-modifying substances with various spectrometric and chromatographic techniques.

3. To synthesize the oviposition-modifying substances, if they are novel and commercially unavailable, by means of modern methods of organic synthesis.

4. To synthesize homologues and analogues of oviposition-modifying substance for procuring more active compounds and for structure-activity relationship studies.

Physical properties and structural chracteristics of these compounds will be used as parameters to correlate their chemical structures with ovipositional activity against several important species of pest and vector mosquitoes.

5. To study ovipositional responses of gravid female mosquitoes to the oviposition-modifying substances in laboratory olfactometers.

6. To investigate the sensory physiology of the oviposition-modifying substances and to identify the locations of chemoreceptors that mediate the perception of the oviposition-modifying substances in mosquitoes.

7. To evaluate the possible toxicity of the oviposition-modifying substances--the toxicity which may possibly influence the accuracy of field evaluations of these behavior-modifying substances.

8. To study the concentration-activity relationship and the species-specificity of the oviposition-modifying substances against various mosquitoes in the genera Culex, Aedes, and Anopheles.

9. To evaluate the oviposition-modifying substances under semi-field conditions using galvanized sheet-metal cylinders in natural mosquito-breeding ponds.

10. To evaluate the oviposition-modifying substances under field conditions using natural mosquito-breeding ponds to assess the effectiveness of these substances in manipulating ovipositional behavior of various species of mosquitoes in the field.

11. To evaluate the feasibility of utilizing the oviposition attractants in traps or light traps for sampling populations of mosquitoes that do not readily respond to conventional mosquito light traps.

E. CURRENT ACCOMPLISHMENTS (SEPT. 1979 - AUGUST 1980)

E-1. STRUCTURE-ACTIVITY RELATIONSHIP AND SPECIES SPECIFICITY OF OVIPOSITION PEPELLENTS

Butyric acid, the major constituent of the active fraction separated from the lab chow infusion, showed a distinctive species-specificity against various species of mosquitoes as reported in the previous annual report. This acid is particularly repellent to An. quadrimaculatus, moderately repellent to Cx. tarsalis and Cx. quinquefasciatus, and less repellent to Ae. aegypti and Cs. incidens.

Of the acids isolated and identified, isobutyric and acetic acids are the most repellent to Cx. quinquefasciatus and caproic acid is most active against Cx. tarsalis. Esters of these acids, such as ethyl acetate, methyl propionate, ethyl propionate, methyl butyrate, and ethyl butyrate, did not exhibit any ovipositional activity against Cx. quinquefasciatus in laboratory olfactometers.

The oviposition repellents are therefore proven to be species specific. They also display different levels of repellency with the changes in their chemical structures. In order to expand this aspect of research and to procure more active compounds, we have carried out the present study. A number of homologous, aliphatic, straight-chain carboxylic acids from C_5 to C_{13} and other compounds or materials were evaluated for their ovipositional repellency against Cx. quinquefasciatus, Cx. tarsalis, and Ae. aegypti.

Methods and Materials. All carboxylic acids, chemical compounds, and materials used in this study were obtained from commercial sources. 3-Methylnonanoic acid was synthesized in this laboratory. An acetone solution of a compound or material was dropped on a disc of filter paper (0.7-mm diameter, Whatman No. 5) until the desired quantity of the acid was impregnated in the paper disc. Acetone was allowed to evaporate, and the paper disc was placed in a Stender dish (37x25 mm with a 4.74-cm^2 surface area) containing 4-ml distilled water. The treated dish, together with a check dish containing a blank paper disc in water, was covered with an inverted 1-liter polystyrene plastic food cup (Amoco No. 41032) and subjected to bioassay test as described in the last annual report (September 1, 1979). For Ae. aegypti testing, a 2.5x11-cm strip of chromatography paper (Whatman No. 1) was placed in the liquid along the inside margin of all Stender dishes to facilitate substrate oviposition of egg by this mosquito.

Five gravid females were introduced into each bioassay unit, and the results of oviposition were recorded after 24 and 48 hours. All tests were replicated at least four times. The criterion for measuring oviposition response was the number of ovipositions in both treatment and the standard. This activity is expressed as the oviposition activity index (OAI) and calculated as follows (Kramer and Mulla, 1979).

$$OAI = \frac{N_t - N_s}{N_t + N_s}$$

N_t denotes the number of ovipositions in a treated sample, and N_s denotes the number of ovipositions in the standard. In Culex, the number of ovipositions was determined from the number of egg rafts observed. In Aedes, the total number of eggs laid during a test is divided by the total number of mosquitoes used to

give the average number of eggs per female. The number of ovipositions was then calculated by dividing the number of eggs in a dish by the average number of eggs per female.

All index values lie within the range from +1 to -1. Positive values indicate that more ovipositions were observed in the treatment than in the standard, indicating the test sample to be attractant in the broad sense. Conversely, more ovipositions in the standard than in the treatment would result in a negative OAI which indicates the test sample is repellent in the broad sense. The data were statistically analyzed, and the significance of all indices was determined by using chi-square analysis with an expected 50-50 ratio in the treatment and the standard.

Results and Discussion. The ovipositional activity of homologous, straight-chain carboxylic acids is presented in Table 1. When tested against Cx. quinquefasciatus, pentanoic, hexanoic, and heptanoic acids showed significant repellency only at the highest 10^{-2}M concentration. Octanoic, nonanoic, decanoic, and undecanoic acids were repellent to this species at lower concentrations. Dodecanoic and tridecanoic acids did not manifest any ovipositional activity at all concentrations tested.

Pentanoic, hexanoic, and undecanoic acids exhibited repellency only at 10^{-2} and 10^{-2}M against Cx. tarsalis, whereas heptanoic, octanoic, nonanoic, decanoic, and dodecanoic acids were repellent at 10^{-2} and 10^{-3}M. Tridecanoic acid showed repellency only at 10^{-3}M.

Undecanoic acid showed repellency only at 10^{-2}M against Ae. aegypti. Pentanoic. hexanoic, octanoic, and decanoic acids were repellent at 10^{-2} and 10^{-3} M and nonanoic acid was repellent at all three concentrations against this species. Although hentanoic acid was significantly repellent at 10^{-2} and 10^{-4} M, it showed a rather low OAI at all concentrations. Dodecanoic acid displayed significant attractancy at 10^{-3} M against this species of mosquito.

In general the common oviposition repellents for the three species of mosquitoes were octanoic, nonanoic, and decanoic acids. They usually showed a higher degree of repellency than the other carboxylic acids. In considering these carboxylic acids as a homologous series of compounds, the oviposition repellency peaked at C_8 to C_{10} acid with the C_9 acid being the highest.

The ovipositional activity of other compounds and materials is listed in Table 2. Skatole, indole, and ammonia are some of the end products of putrefaction of organic matter. Skatole showed significant repellency at all concentrations against the gravid females of Cx. quinquefasciatus. Indole exhibited repellency only at the highest concentration whereas ammonia did not show any measurable activity at all concentrations. High concentrations of ammonia were reported to repel Ae. albopictus and Ae. polynesiensis (Gubler 1971).

Alkanols are the precursors of alkanoic acids in fermentation of organic matter. 1-Octanol (not listed) was not active at concentrations from $7.7X10^{-6}$ to $7.7x10^{-4}$ M whereas 1-hexadecanol showed ovipositional repellency at $4.1X10^{-4}$ and $4.1X10^{-5}$ M.

2-Methylnonanoic acid, a branched-chain structural isomer of decanoic acid, was as repellent as decanoic acid showing the lowest effective concentration at 10^{-3} M. In this case, the carbon-chain branching did not seem to affect the level of repellency between the isomeric decanoic acids.

Commercially available contact insect repellents were also evaluated for their ovipositional repellency. MGK-11 and "OFF" (a mixture of Delphene, MGK-11, and MGK-264) were repellent only at 100 ppm whereas Delphene was ovipositionally attractive at the same concentration. Dibutyl phthalate did not show any activity at all concentrations. It seems that the repellency exhibited by the commercially available repellents does not bear any direct relationship with their ovipositional activity.

Among the miscellaneous compounds and materials, skatole, 1-hexadecanol, and 2-methylnonanoic acid were relatively active. Further studies seem to be necessary to explore these types of compounds more thoroughly.

E-2. SEMI-FIELD EVALUATIONS OF OVIPOSITION REPELLENTS

Lower aliphatic carboxylic acids were found to be oviposition repellents for several species of mosquitoes in laboratory bioassay tests (Hwang et al. 1980). Further studies showed that their higher homologues, particularly C_8, C_9, and C_{10} acids, exhibited higher levels of repellency against Culex and Aedes mosquitoes. The structure-activity relationship and the effective concentrations of these higher acids were investigated and are reported in the preceding section. To understand the working mechanism of the repellents and to explore the practicality of using them in the field, we conducted a series of exploratory semi-field experiments. The data and information obtained in the semi-field studies would provide a firm basis for conducting further full-scale field studies.

Methods and Materials. As reported in the preceding section and described in Table 1, octanoic, nonanoic, and decanoic acids are generally the most effective repellents. In addition, they are not as odoriferous as their lower homologues. In this regard, one of these acids seemed to be the compound of choice for the semi-field evaluation.

The evaluation was conducted at the Aquatic Research Facility at the University of California, Riverside. Four rectangular field ponds, 3.6X7.2 m each, were used in this study. Four metal-sheet cylinders, each with 46-cm height and 43- to 47-cm diameter, were tightly secured at the bottom of each pond. Each cylinder was filled with pond water. The top edges of the cylinders were a few centimeters higher than the water level in the ponds so that water in the cylinders would be isolated from the rest of the ponds.

Two experiments were carried out. In the first experiment, the cylinders were grouped into four treatments.

Treatment 1. Containing chicken manure (0.2%) and octanoic acid (caprylic acid, 6 ppm).

Treatment 2. Containing chicken manure (0.2%) and octanoic acid (30 ppm).

Treatment 3. Containing only chicken manure (0.2%).

Treatment 4. Check (blank).

In the second experiment, the procedures in the first experiment were followed except that Treatment 1 contained 0.2% of chicken manure and 15 ppm of octanoic acid.

Previously, we reported that a chicken manure infusion was attractive to gravid female mosquitoes in the laboratory (Kramer and Mulla 1979) and in the field (see Section E-4). In evaluating oviposition repellents, we assumed that mosquito oviposition would be rather sparse. Therefore, it would be difficult to evaluate the repellent accurately. To amend this situation, we decided to spike the cylinders with chicken manure which would facilitate greater mosquito oviposition in the cylinders.

Each pond therefore contained four cylinders with four different treatments. The cylinders were placed in randomly arranged fashions to prevent positional effects. The cylinders were first treated with chicken manure one day after flooding the ponds, and, three days after flooding, octanoic acid was added into the cylinders in the first experiment. In the second experiment, chicken manure was added one day after flooding, and octanoic acid was added five days after flooding. After flooding, egg rafts were collected from the cylinders every day for 28 days in the first experiment and for 22 days in the second experiment. The egg rafts were brought to the laboratory for counting and reared until adult emergence for species identification.

Results and Discussions. Results of the first experiment are illustrated in Figure 1, in which the cumulative mean number of egg rafts per cylinder in each treatment is plotted against the number of days after flooding the ponds. Twenty-five days after the addition of octanoic acid, the cumulative mean number of egg rafts per cylinder in Treatment 2 cylinders (0.2% chicken manure and 30 ppm octanoic acid) was only about 33, whereas that in Treatment 4 cylinders (check) was 87. In Treatment 3 cylinders (0.2% chicken manure only), a total of 144 egg rafts was found. In Treatment 1 cylinders (0.2% chicken manure and 6 ppm octanoic acid), there were as many as 219 egg rafts.

From these results, we concluded that the chicken manure infusions, at the 0.2% concentration, increased the ovipositional activity of mosquitoes in the cylinders whereas octanoic acid, at the 30 ppm concentration, suppressed the ovipositional activity of mosquitoes. At the lower 6 ppm concentration, octanoic acid was not sufficient to cancel the attractiveness of the chicken manure and thus failed to suppress the female mosquitoes from ovipositing.

Figure 2 illustrates the results obtained in the second experiment. Seventeen days after the addition of octanoic acid, the accumulative mean number of egg rafts in Treatment 1 cylinders (0.2% chicken manure and 15 ppm octanoic acid) was only 34, and the number in Treatment 2 cylinders (0.2% chicken manure and 30 ppm octanoic acid) was 75. In Treatment 4 cylinders (check), an average of 111 egg rafts was found. In Treatment 3 cylinders (0.2% chicken manure), an average of 165 egg rafts was deposited. The findings not only reconfirmed the previous conclusions but also provided new information that octanoic acid was capable of suppressing mosquito oviposition at the 15 ppm concentration.

Mosquitoes collected from the cylinders were mainly Cx. tarsalis and Cx. peus Speiser.

As a result of these studies we have been able to extend our knowledge obtained in the laboratory to the semi-field situations. We have thus proven that the aliphatic carboxylic acids manifest ovipositional repellency against gravid mosquitoes under laboratory as well as under semi-field conditions.

E-3. FIELD EVALUATION OF OVIPOSITION REPELLENTS

The oviposition repelllents of mosquitoes developed in this laboratory demonstrated their effectiveness both in laboratory olfactometers and in olfactory cylinders under semi-field conditions. The laboratory evaluation of the oviposition repellents was carried out under controlled conditions with confined spaces in the olfactometer, regulated air flow, and limited areas on which the repellents were applied and mosquitoes oviposited. Although natural factors, such as weather, greatly affected the outcome of the semi-field evaluation, the oviposition repellents were again confined in limited areas of water in the cylinders with rather uniform distribution of the repellents on the cylinder surfaces. The full-scale field studies would be more difficult to achieve because of numerous uncontrollable factors involved. In employing the oviposition repellents in vast areas of natural habitats of mosquitoes, dosage, evaporation rate, and distribution of the compounds must be taken into account. Here, we report our findings in this study.

Methods and Materials

Experiment 1. The first experiment was conducted in our Aquatic Research Facility located in Oasis, California. Sixteen experimental ponds, 4.8X4.8 m each, were randomly categorized into four groups with four ponds in a group. Three days after flooding the ponds, the first and the second groups of ponds were treated, respectively, with 10 and 20 ppm of octanoic acid formulated in the 1:1 ratio with 1-propanol. The third group of ponds was treated with the equal amount of 1-propanol as in the 20-ppm treatments, and the fourth group was kept untreated as checks. The repellent was applied in the ponds by spraying it with 500-ml washing bottle. This experiment therefore consisted of four treatments with four replicates in each treatment. For larval assessment, water samples (five dips per pond) were taken from the ponds 0, 4, and 6 days after the treatment and brought to the laboratory for counting and species identification.

Experiment 2. This experiment was carried out in the Aquatic Research Facility on the Riverside Campus of the University of California. Twelve experimental field ponds, 3.6X7.2 m each, were randomly categorized into four groups with three ponds in each group. Immediately after flooding the ponds, the first and the second groups of the ponds were treated, respectively, with 25 and 50 ppm of octanoic acid formulated in the 1:1 ratio with isopropyl alcohol. The dosages used in Experiment 1 were found to be insufficient; therefore, in this experiment, the concentrations were increased. The third group was treated with the equal amount of isopropyl alcohol as in the 50-ppm treatments, and the fourth group was kept untreated as checks. The repellent was sprayed in 500-ml washing bottles as in the previous experiment. This experiment thus consisted of four treatments with three replicates in each treatment. Every 2-3 days, for larval assessment, water samples were collected and counted. Having found that octanoic acid was not effective in repelling mosquitoes from oviposition eight days after the treatment, we decided to conduct another treatment in order to ascertain its effectiveness. Therefore, nine days after the treatment, the ponds were treated again in the same manner as in the previous treatment.

Experiment 3. In this experiment, nonanoic acid was used instead of octanoic acid. Both acids showed a similar level of repellency against Cx. tarsalis in the laboratory olfactometers. A formulation was made by mixing 80 volume parts of nonanoic acids, 20 volume parts of 1-propanol, and 3 volume parts of Triton X-100. The surface active agent was used for obtaining relatively even distribution of the repellent on the water surface. The repellent formulation was sprayed onto the ponds with 1-gallon hand sprayers. A total of ten experimental ponds located at the Aquatic Research Facility on this campus was used; three ponds were treated with the repellent formulation equivalent to 150 ppm nonanoic acid; three ponds were treated with the equal amounts of 1-propanol and Triton X-100 as in the above-mentioned 150-ppm treatment; the four remaining ponds were kept untreated as checks. The dosages used in Experiment 2 were found to be still insufficient. It was therefore necessary to increase the concentration of nonanoic acid to 150 ppm. Samples were taken from the ponds from time to time and the collected immature mosquitoes were counted. The numbers of egg rafts in the ponds were also counted at the same time.

Results and Discussions

Experiment 1. The pretreatment sampling showed no difference among the numbers of first-instar larvae from the four groups of ponds (Table 3). Four days after the treatment, the samples mainly consisted of first and second instars. Although there was some repellency shown by the 20-ppm treatments and the solvent checks as compared with the checks, the difference was not significant. Third and fourth instars started to appear six days after the treatment. Some reductions were observed in the numbers of first and second instars in the treated ponds and the solvent checks; however, the difference was again not significant. It was therefore obvious that octanoic acid, at the 10 and 20 ppm concentrations, did not demonstrate any ovipositional activity under field conditions. The reason for this failure might be due to the low dosages employed in this experiment.

Experiment 2. In this experiment, the concentrations were increased to 25 and 50 ppm. The results obtained in the field tests are presented in Table 4. Up to eight days after the treatment, the numbers of mosquito larvae collected from either the 25-ppm or 50-ppm treated ponds did not show any significant difference from those collected from either the solvent checks or the checks; therefore, a re-treatment became necessary.

Nine days after the treatment, the ponds were treated again in the same manner as in the previous treatment. Three days later only the number of second instars collected from the 25-ppm treatments was significantly lower than that of the checks. The numbers of first, third, and fourth instars from both treatments, the solvent checks, and the checks were not statistically different although some insignificant repellencies were observed sporadically.

Sixteen days after the first treatment and seven days after the second treatment, the numbers of mosquito larvae from the treated ponds were as many as those from the control ponds. The mosquitoes collected from the ponds were mainly Cx. tarsalis and Cx. peus.

From the data obtained from this field study, we concluded that octanoic acid at the 25 and 50 ppm concentrations failed to repel mosquitoes from oviposition under the field conditions. The low dosages and the uneven distribution of the repellent on the water surface in the experimental ponds might have caused the low repellent activity of this acid.

Experiment 3. The effectiveness of nonanoic acid, as a formulation with 1-propanol and Triton X-100, is presented in Table 5. With a few exceptions, the numbers of egg rafts in the treated ponds were lower than those in the checks throughout the experiment; up to six days after the treatment, the differences were significant. The numbers of first-, second-, third-, and fourth-instar larvae and pupae in the treated ponds were lower than those in the checks up to 14, 14, 14, 17, and 24 days, respectively, after the treatment. Due to the limited number of replicates in this experiment, some of the differences among the numbers were not statistically significant. Nonetheless, nonanoic acid seemed to show oviposition repellency under the experimental conditions described.

Recently, we are carrying out studies on the repellency of various nonanoic acid formulations. In one of the studies, we have found that 75 ppm of nonanoic acid formulated with xylene and a Triton surfactant shows good oviposition repellency. Further studies are underway and will be reported in the future.

E-4. FIELD EVALUATION OF ATTRACTIVE ORGANIC INFUSIONS

Previously, we reported that a 1% chicken manure infusion, fermented for 9 to 26 days, was significantly attractive to the gravid females of Cx. quinquefasciatus in laboratory bioassay tests (Kramer and Mulla 1979). For ascertaining the effectiveness of this infusion under field conditions, the present study was undertaken.

Methods and Materials. The field evaluation of the chicken manure infusion was conducted in the natural field ponds at the Aquatic and Vector Control Research Facility in Oasis, California. Eight ponds were used for this purpose. Each pond had a measurement of 4.8X4.8 m and held 10,000 liters of water. Four ponds, randomly selected, were treated with chicken manure at the 1% concentration, and the other four ponds were kept untreated as control. Samples were taken every 3-4 days and brought back to the laboratory for counting and for classifying the immature mosquitoes in the samples.

Results and Discussion. The mean numbers of immature mosquitoes collected from both treated and control ponds are listed in Table 6. Each mean from the treated ponds was invariably higher than that from the control ponds. The chicken manure infusion was therefore effective in attracting female mosquitoes for oviposition. The mosquitoes collected from these ponds were predominantly Cx. tarsalis with a small number of Culiseta inornata (Williston).

E-5. ISOLATION AND IDENTIFICATION OF OVIPOSITION ATTRACTANTS

In the previous annual report, we reported our efforts in isolating the oviposition attractants in semi-pure forms for chemical identification. The attractive chicken manure infusion was thus steam-distilled under atmospheric pressure,

and the distillate was extracted with ether. The ether extract was found to be attractive in laboratory bioassay tests.

For the past year, our endeavors were placed on the further purification and identification of the oviposition attractants. In this section, we report our procedures and results of this aspect of research.

Methods and Materials. The chicken manure infusion (120 liters), fermented for nine days, was subjected to steam distillation to yield a distillate (240 liters). The distillate, 1 liter at a time, was extracted with ether (1X300 ml, 2X200 ml), and the combined extracts were dried over Na_2SO_4 and evaporated to dryness. The residual ether extract was subjected to bioassay tests.

The ether extract (2.4 g) was again dissolved in ether (100 ml) and the ether solution was extracted with 5% aqueous NaOH (3X30 ml). The aqueous layers were combined, washed with ether (1X30 ml), and acidified with conc. HCl to pH 3-4. The acidified solution was extracted with ether (3X50 ml). The combined ether extracts were washed with water (1X10 ml) and dried over Na_2SO_4. Evaporation of the ether solution resulted in obtaining an acidic fraction (0.07 g).

The ether solution, after the separation of the acidic fraction, was then extracted with 5% HCl (3X30 ml). The ether layer was washed with water, dried over Na_2SO_4, and evaporated to give a neutral fraction (1.27 g). The aqueous layers were combined, washed with ether (1X30 ml), and basified with 5% aqueous NaOH to pH 9-10. The basified solution was extracted with ether (3X50 ml). The ether solutions were combined, washed with water (1X10 ml), dried over Na_2SO_4, and evaporated to give a basic fraction (0.08 g). All these three fractions were bioassayed.

The neutral fraction was analyzed on a silica-gel, thin-layer plate developed two-dimensionally first with heptane-benzene (1:1) mixture and then with cyclohexane. This fraction was also analyzed with a gas chromatograph with a 10% Apiezon L on Chromosorb W column.

The neutral fraction (1.04 g) was chromatographed on silica gel (60 g) and eluted successively with solvents with increasing polarity. The solvent system used in this procedure was in the order of petroleum ether, 50% petroleum ether and 50% cyclohexane, cyclohexane, 50% cyclohexane and 50% benzene, benzene, 50% benzene and 50% dichloromethane, and dichloromethane (300 ml of each solvent or mixture of solvents). The eluate was collected in 50-ml aliquot. A total of 43 fractions was collected. The composition of each fraction was monitored by gas chromatograph with an Alltech CS-10 column, an SE-30 column, and an Apiezon L column. Fractions with similar compositions were combined into 11 fractions (from Fraction A through Fraction K). Each fraction was subjected to bioassay tests.

Infrared spectra of each fraction were taken with a Perkin-Elmer model 727 infrared spectrophotometer. Nuclear magnetic resonance spectra were taken with a Varian model EM-390 90 MHz nmr spectrometer.

Two gas chromatography-mass spectrometer (GC-MS) systems were used to analyze the fraction thus obtained. The first GC-MS system consisted of a Varian Aerograph model 1400 gas chromatograph interfaced with a Finnigan model

3100 mass spectrometer. An Alltech CS-10 column was used in the gas chromatograph. The second GC-MS system used was a Varian Aerograph model 1400 gas chromatograph (with a CS-10 column) interfaced with a Finnigan model 1015 mass spectrometer attached to a System 150 data acquisition and reduction system.

During the isolation procedure, the bioassay method described in Section E-1 was used to monitor the activity of each fraction.

Results and Discussion. The chicken manure infusion, upon steam distillation, yielded an attractive distillate which was extracted with ether to give an attractive ether extract (see the annual report, September 1, 1979). The ether extract was fractionated to give a neutral, an acidic, and a basic fraction. The ovipositional activity of these three fractions is listed in Table 7. The basic fraction showed significant oviposition repellency against Cx. quinquefasciatus at the $2X10^{-4}$% concentration whereas the acidic fraction did not exhibit any activity within the concentrations tested. The neutral fraction showed attractancy at the $2X10^{-4}$ and $2X10^{-3}$% concentrations. It was apparent that the neutral fraction contained the oviposition attractants.

The active neutral fraction showed six spots on a silica-gel, thin-layer plate. On gas chromatographic analysis on an Apiezon column, this fraction gave five minor peaks and a major peak.

Upon column chromatography on silica gel, the neutral fraction gave a major component eluted by a cyclohexane-benzene mixture (1:1). The major component consisted of fractions designated as Fractions E, F, G, and H, which showed only one major peak in gas chromatographic analysis. This major component gave nmr signals at δ0.94, 1.25, 3.45, 4.12, and 7.57 ppm and maximum ir absorption at 1735, 1605, 1583, 1470, 1395, 1290, 1130, 1080, and 755 cm^{-1}. The spectrometric data suggested that there were ester linkages, an ortho-substituted benzene ring, and two tertially substituted carbons β to carboxyl functional groups.

Fraction A, presumably a mixture of hydrocarbons, did not show any activity in laboratory bioassay tests (Table 7). Fractions E through H constituted the major component in the neutral fraction. Fraction E displayed oviposition attractancy at $7.25X10^{-3}$%, Fraction F at $4.5X10^{-3}$%, and Fraction H at $1X10^{-3}$ and $1X10^{-2}$%. At $4.5X10^{-5}$%, Fraction F showed significant repellency. Fraction G was not active within the concentrations used for bioassays.

The major component (Fractions E through H) gave a mass spectrum with major peak at m/e 279, 167, 150, 149 (base peak), 113, and 104 in the first GC-MS system. In the second GC-MS system, mass spectra were scanned every 20 seconds. The total ion current was plotted against the spectrum number to give a reconstructed gas chromatogram which showed the presence of a minor peak and a major peak. The minor peak showed m/e at 149, 147, 129, 113, 112, 111, 97, 91, 87, 84, 83, 82, 81, 73, 71, 70, 69, 67, 57, 56, 55, 44, 43, 42, 41, 40, 39, 32, 29, 28 (base peak), and 18. The major peak gave m/e at 279, 168, 167, 150, 149 (base peak), 113, 104, 83, 76, 71, 70, 69, 57, 56, 55, 44, 43, 41, 29, 28, and 27.

The mass spectrometric data strongly suggested the possible presence of a phthalic ester in the major component of the neutral fraction. More work is underway to affirmatively confirm the structure. At the same time, a special consideration is given to determine whether this compound is derived from natural products or an artifact introduced during the process of isolation.

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Table 1. The ovipositional activity of straight-chain aliphatic carboxylic acids against Culex and Aedes mosquitoes.

Acid	M	Oviposition Activity Index[a/]		
		Cx. quinquefasciatus	Cx. tarsalis	Ae. aegypti
Pentanoic	10^{-2}	-0.93**	-0.90**	-0.96**
	10^{-3}	+0.03	-0.24	-0.29**
	10^{-4}	+0.04	-0.03	-0.19
Hexanoic	10^{-2}	-1.00**	-1.00**	-0.98**
	10^{-3}	-0.08	-0.23	-0.47**
	10^{-4}	0	-0.09	+0.02
Heptanoic	10^{-2}	-1.00**	-1.00**	-0.42**
	10^{-3}	0	-0.75**	-0.34
	10^{-4}	+0.07	-0.11	-0.35*
Octanoic	10^{-2}	-1.00**	-1.00**	-1.00**
	10^{-3}	-0.76**	-1.00**	-0.44**
	10^{-4}	-0.06	-0.40	-0.21
Nonanoic	10^{-2}	-1.00**	-1.00**	-1.00**
	10^{-3}	-0.89**	-0.90**	-0.67**
	10^{-4}	-0.33*	0	-0.81**
	10^{-5}			-0.37*
Decanoic	10^{-2}	-1.00**	-1.00**	-0.99**
	10^{-3}	-0.50*	-1.00**	-0.44*
	10^{-4}	-0.12	-0.23	+0.12
Undecanoic	10^{-2}	-1.00**	-1.00*	-1.00**
	10^{-3}	-0.72**	-0.45	+0.12
	10^{-4}	-0.02	0	+0.25
Dodecanoic	10^{-2}	-0.27	-1.00**	-0.20
	10^{-3}	-0.24	-1.00*	+0.42**
	10^{-4}	-0.20	-0.50	-0.01
Tridecanoic	10^{-2}	-0.13	+0.33	0
	10^{-3}	+0.09	-0.82**	+0.06
	10^{-4}	-0.08	+0.25	+0.25

a/**Significant at P<0.01. *Significant at P<0.05.

Table 2. The ovipositional activity of miscellaneous compounds and materials against Cx. quinquefasciatus.

Material	Concn. (M or ppm)	OAI[a/]
Skatole	7.6×10^{-4} M	-0.94**
	7.6×10^{-5}	-0.40*
	7.6×10^{-6}	-0.40*
Indole	8.5×10^{-4} M	-0.94**
	8.5×10^{-5}	-0.22
	8.5×10^{-6}	-0.02
1-Hexadecanol	4.1×10^{-4} M	-0.89**
	4.1×10^{-5}	-0.62**
	4.1×10^{-6}	-0.05
2-Methylnonanoic acid	1×10^{-2} M	-1.00**
	1×10^{-3}	-0.71**
	1×10^{-4}	-0.16
MGK-11	100 ppm	-0.78**
	10	+0.20
	1	+0.17
Delphene	100 ppm	+0.44*
	10	+0.20
	1	-0.36
"OFF"	100 ppm	-0.85*
	10	+0.25
	1	-0.13
Dibutyl phthalate	3.6×10^{-4}	-0.33
	3.6×10^{-5}	+0.12
	3.6×10^{-6}	-0.20
NH_4OH	1×10^{-2} M	-0.18
	1×10^{-3}	+0.10
	1×10^{-4}	0
	1×10^{-5}	-0.12

a/**Significant at $p<0.01$. *Significant at $p<0.05$.

Table 3. Field evaluation of octanoic acid as oviposition repellent in Oasis, California (Experiment 1).

Days After Flooding	Days After Treatment	Concn (ppm)	Average No. of Larvae per Pond & % Repellency[a/]							
			1st		2nd		3rd		4th	
			No.	% Rep.	No.	% Rep.	No.	% Rep.	No.	% Rep.
3	0	10	8a	0	0	0	0	0	0	0
	(Pretreatment)	20	5a	0	0	0	0	0	0	-
		Solvent Check	5a	0	1	0	0	0	0	0
		Check	3a	-	0	-	0	-	0	-
7	4	10	64a	0	16a	0	0	0	0	0
		20	34a	51	8a	20	0	0	0	0
		Solvent Check	56a	19	8a	20	1	0	0	0
		Check	69a	-	10a	-	0	-	0	-
9	6	10	62a	7	33a	0	14a	0	6a	0
		20	56a	16	16a	27	8a	0	3a	0
		Solvent Check	48a	28	24a	0	10a	0	3a	0
		Check	67a	-	22a	-	4a	-	1a	-

[a/] Means based on 4 replicates. Means with the same letters are not significant in the analysis of variance and in the Duncan's multiple range test.

Table 4. Field evaluation of octanoic acid as oviposition repellent in the Aquatic Research Facility, UCR (Experiment 2).

Days After Flooding	Days After Treatment	Concn (ppm)	Average No. of Larvae per Pond & % Repellency[a/]							
			1st		2nd		3rd		4th	
			No.	% Rep.	No.	% Rep.	No.	% Rep.	No.	% Rep.
1	1	25	0	-	0	-	0	-	0	-
		50	0	-	0	-	0	-	0	-
		Sol. Check	0	-	0	-	0	-	0	-
		Check	0	-	0	-	0	-	0	-
2	2	25	0	-	0	-	0	-	0	-
		50	0	-	0	-	0	-	0	-
		Sol. Check	0	-	0	-	0	-	0	-
		Check	0	-	0	-	0	-	0	-
5	5	25	22a	0	0a	0	0	-	0	-
		50	15a	32	2a	0	0	-	0	-
		Sol. Check	44a	0	0a	0	0	-	0	-
		Check	22a	-	2a	-	0	-	0	-
8	8	25	169a	0	94a	0	6a	0	0	-
		50	100a	0	42a	0	5a	0	1	-
		Sol. Check	247a	0	121a	0	7a	0	0	-
		Check	42a	-	17a	-	2a	-	0	-
9	9	Retreatment								
12	3	25	122a	0	24c	59	4a	81	3a	79
		50	101a	0	38bc	34	6a	72	5a	64
		Sol. Check	72a	0	94a	0	15a	29	9a	36
		Check	66a	-	58ab	-	21a	-	14a	-
16	7	25	31a	40	47a	0	26a	0	20a	20
		50	82a	0	110a	0	44a	0	21a	16
		Sol. Check	66a	0	82a	0	24a	0	26a	0
		Check	52a	-	41a	-	17a	-	25a	-

[a/] Means based on 3 replicates. Means with the same letters are not significant in the analysis of variance and in the Duncan's multiple range test.

Table 5. Field evaluation of nonanoic acid as oviposition repellent in the Aquatic Research Facility, UCR (Experiment 3).

Days After Flooding	Days After Treatment	Concn (ppm)	Average No. of Egg rafts, Larvae, and Pupae and % Repellency[a/]											
			Egg Raft		1st		2nd		3rd		4th		Pupae	
			No.	% Rep.	No.	% Rep.	No.	% Rep.	No.	% Rep.	No.	% Rep.	No.	% Rep.
1	0	150	1.3a	78	-	-	-	-	-	-	-	-	-	-
		Sol. Check	3.3a	45	-	-	-	-	-	-	-	-	-	-
		Check	6.0a	-	-	-	-	-	-	-	-	-	-	-
2	1	150	0a	100	-	-	-	-	-	-	-	-	-	-
		Sol. Check	7.7a	6	-	-	-	-	-	-	-	-	-	-
		Check	8.2a	-	-	-	-	-	-	-	-	-	-	-
3	2	150	0b	100	-	-	-	-	-	-	-	-	-	-
		Sol. Check	8.3a	72	-	-	-	-	-	-	-	-	-	-
		Check	29.2a	-	-	-	-	-	-	-	-	-	-	-
4	3	150	0b	100	0a	100	0a	100	0	-	0	-	-	-
		Sol. Check	10.3b	81	19a	41	4a	0	0	-	0	-	-	-
		Check	55.2a	-	32a	-	4a	-	0	-	0	-	-	-
7	6	150	9.0b	90	5b	98	0b	100	0b	100	0a	100	-	-
		Sol. Check	26.3ab	72	57ab	80	11b	87	10ab	9	4a	0	-	-
		Check	92.5a	-	279a	-	83a	-	11a	-	4a	-	-	-
10	9	150	14.0a	81	62a	74	25a	88	11a	85	2a	94	0a	100
		Sol. Check	40.7a	45	573a	0	194a	10	62a	14	19a	44	8a	0
		Check	73.5a	-	236a	-	215a	-	72a	-	34a	-	8a	-
15	14	150	43.7a	7	359a	18	104a	15	18a	82	32a	61	6a	87
		Sol. Check	35.3a	34	621a	0	137a	0	86a	12	58a	29	38a	17
		Check	53.5a	-	440a	-	123a	-	98a	-	82a	-	46a	-
18	17	150	58.7a	0	137a	0	162a	0	53	0	14a	58	8a	53
		Sol. Check	42.3a	18	145a	0	211a	0	33a	8	48a	0	42a	0
		Check	51.5a	-	112a	-	83a	-	36a	-	33a	-	17a	-
25	24	150	5.0a	65	50a	0	138a	0	29a	0	24a	0	36a	14
		Sol. Check	10.3a	28	26a	0	22a	65	9a	50	11a	8	7a	0
		Check	14.2a	-	18a	-	62a	-	18a	-	12a	-	42a	-
30	29	150	72 a	0	389a	0	93a	0	111a	0	105a	0	70a	0
		Sol. Check	21 a	16	228a	40	20a	47	4b	80	5a	30	4a	20
		Check	25 a	-	380a	-	38a	-	20b	-	30a	-	5a	-

[a/] Means based on 3 replicates for the treated and the solvent checks and 4 replicates for the checks. Means with the different letters are significantly different from one another.

Table 6. Field evaluation of 1% chicken manure infusion in Oasis, California

Days After Treatment	Mean No. of Immature Mosquitoes[a/]	
	Treated	Control
4	61	40
8	230	98
11	229	106
14	281	62
21	238	123

[a/] Means based on 4 replicates.

Table 7. Oviposition activity indices of fraction obtained from chicken manure infusion against Cx. quinquefasciatus.

Fraction	Concn (%)	OAI[a/]
Basic	$2x10^{-5}$	-0.14
	$2x10^{-4}$	-0.53**
	$2x10^{-3}$	+0.23
Acidic	$1.75x10^{-5}$	+0.29
	$1.75x10^{-4}$	0
	$1.75x10^{-3}$	-0.23
Neutral	$2x10^{-5}$	-0.14
	$2x10^{-4}$	+0.32*
	$2x10^{-3}$	+0.70**
	$2x10^{-2}$	-0.17
A	$1x10^{-5}$	+0.12
	$2x10^{-5}$	+0.19
	$4x10^{-5}$	0
E	$7.25x10^{-4}$	+0.06
	$7.25x10^{-3}$	+0.65**
	$7.25x10^{-2}$	+0.28
F	$4.5\ x10^{-5}$	-0.40*
	$4.5\ x10^{-4}$	0
	$4.5\ x10^{-3}$	+0.53**
G	$1x10^{-4}$	-0.23
	$1x10^{-3}$	+0.13
	$1x10^{-2}$	+0.27
H	$1x10^{-4}$	0
	$1x10^{-3}$	+0.50*
	$1x10^{-2}$	+0.62*

[a/] *Significant at $p<0.05$

**Significant at $p<0.01$

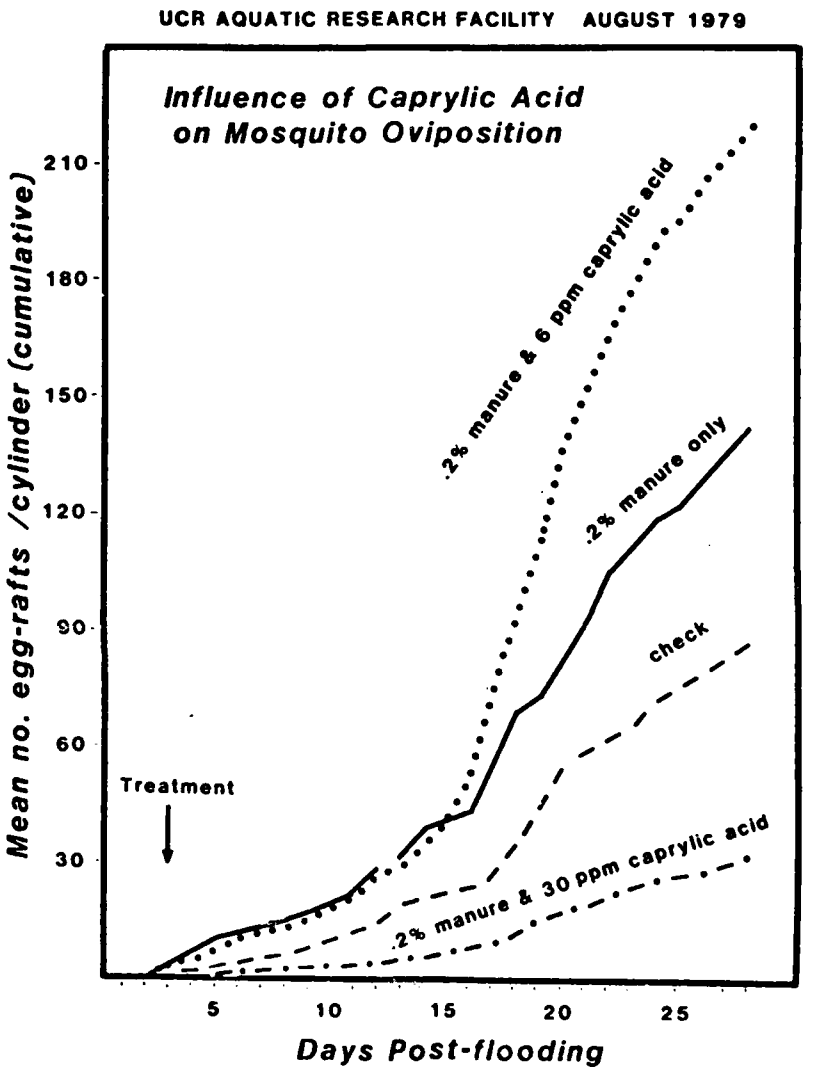

Figure 1. The ovipositional repellency of octanoic acid at 6 and 30 ppm under semi-field conditions.

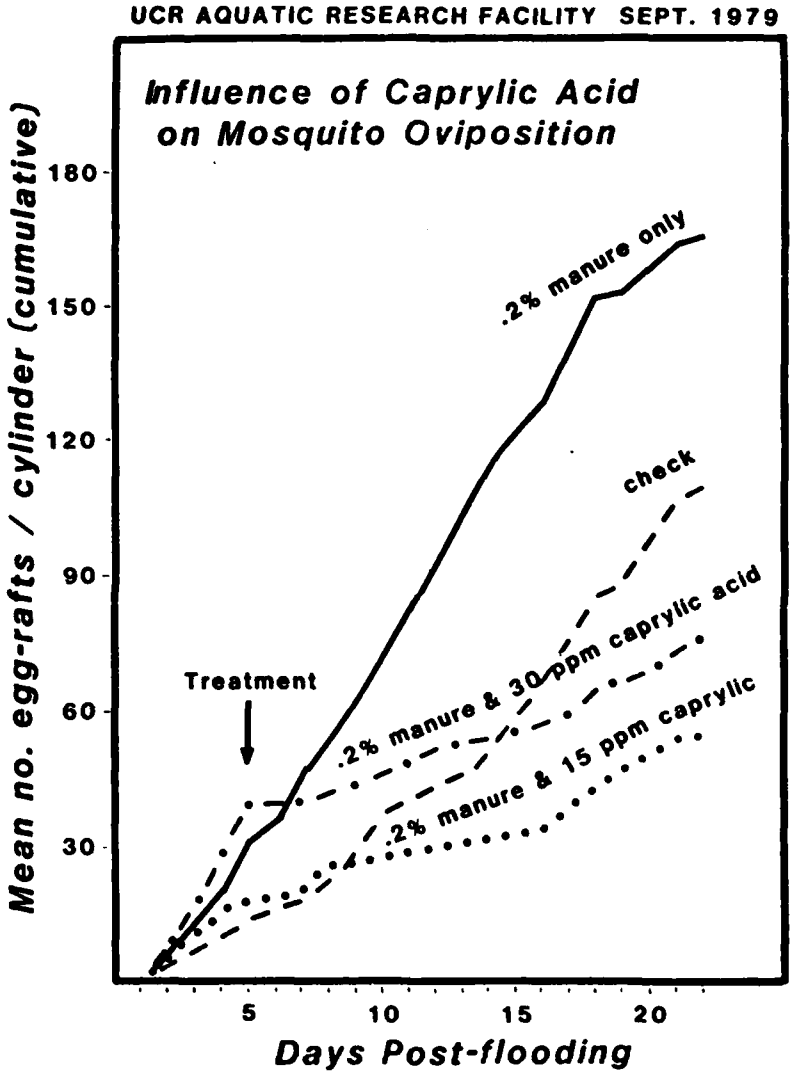

Figure 2. The ovipositional repellency of octanoic acid at 15 and 30 ppm under semi-field conditions.

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